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13. ABSTRACT (Maximum 200 Words)

Huanglian is a botanical prepared from the root of Coptis chinensis and deltoidea. We reported that huanglian inhibits growth of breast cancer cells in vitro in a dose-dependent manner (Li X. et al. Molecular Pharmacology, 58: 1287-1293, 2000). Based on these results, we developed huanglian, as an herbal extract, packaged in 250 mg capsules suitable for human clinical trials. The specific aims of the grant are to (1) conduct a phase I clinical trial of huanglian and (2) conduct a phase II clinical trial of huanglian in patients with chemotherapy refractory breast cancer. We have treated 22 patients on the phase I trial of huanglian. We observed grade 3 diarrhea, but we have yet to achieve an MTD. However, because of capsule number (33) at the highest dose tested (8.25 gm/day), it became impossible for patients to comfortably receive higher doses of the herb. Therefore, we placed the study on hold until we could identify a new source of root from China that would meet our required "biochemical" profile. We are in the process of identifying such a source so as to complete the phase I clinical trial and conduct the phase II clinical trial in breast cancer.

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Gary K. Schwartz, MD
Huanglian: Annual Review

Introduction

Huanglian is a botanical agent prepared as a tea from the roots of Coptis chinensis. In traditional Chinese medicine it has been used to treat inflammatory conditions ranging from gastroenteritis to acute febrile illnesses with no reported toxicity. We tested huanglian for activity against cancer at MSKCC. We reported that huanglian potently inhibits the growth of a number cancer cells in vitro in a dose-dependent manner, with maximal inhibition at low micromolar concentrations (Li X. et al. Molecular Pharmacology, 58:1287-1293, 2000). MCF-7 and MDA-468 breast cancer lines were particularly sensitive to huanglian. The activity of huanglian was greater than an equivalent concentration of its major component, berberine, suggesting that several components contribute to its anticancer effect. It was therefore decided to take whole huanglian to human trial, as a novel departure from the conventional approach in drug development in which a single active compound is selected and tested. In addition to single agent activity against breast cancer cell lines, huanglian was also shown to enhance the effect of paclitaxel, supporting the future development of huanglian in combination with paclitaxel for the treatment of patients with metastatic breast cancer.

Research Aims/Key Research Accomplishments

The overall goal for this grant is to develop new therapeutic approaches in the treatment of patients with metastatic breast cancer based utilizing the Chinese botanical huanglian. The specific aims are to:

1) To conduct a phase I clinical trial of huanglian with both toxicity and efficacy endpoints.
2) Based on the results of the phase I clinical trial of single agent huanglian, conduct a phase II clinical trial of huanglian either as a single agent or in combination with paclitaxel in the treatment of patients with metastatic breast cancer (planned for 2005).

Reportable Outcomes

1. "A Phase I Study of the Chinese Herb Huanglian (Coptis chinesis) in Patients with Advanced Solid Tumors" (MSKCC Protocol Number 00-061A(6): The purpose of this phase I study is to determine the optimal dose of huanglian for future phase II trials. Patients with advanced solid tumors who have failed all conventional therapy or for which there is no conventional therapy are eligible for this study. Twenty-one patients have been registered to this study. One patient elected to withdraw consent after study registration and never received huanglian.

i) Study design, defining the MTD, and best response: The initial study design utilized a rapid dose escalation schedule of 1 patient/level and the huanglian dose was to be increased by 50% in successive cohorts. The starting dose of huanglian was 1 gm/day or one capsule (250 mg/tablet), p.o., 4x/day. At does level 3 (2.25 gm/day), one additional patient was added since the first patient developed progression of disease (POD) before

completing her assessment for toxicity. Using this study design, we safely escalated to a dose of 3.5 gm/day or 14 capsules in 4 divided doses. At a dose of 5.25 gm/day (21 capsules/day), one patient developed grade 3 diarrhea (DLT) and the cohort was expanded to 6 patients with no further DLTs noted. However, because of this toxicity, the study design now changed to a classic dose escalation schema of 3 to 6 patients/dose level and a 25% dose escalation in all successive cohorts. Utilizing this approach, we escalated to a dose of 6.56 gm/day (26 capsules/day) in 3 patients without DLT. In the next cohort of 8.25 gm/day (33 capsules/day), we again observed 1 patient with grade 3 diarrhea. This cohort was expanded to 6 patients and no other DLTs were observed. These results are summarized in the table below, which also indicates stable disease as best response in several patients on the study, including 1 patient with metastatic breast cancer. Each of these patients had been progressing under observation or on therapy before entering the clinical trial with huanglian.

Cohort	Pt. #	Dose (gm/day)	Pill #	Toxicity	Best Response
1	1	1	4	0	Stable (colon): 6.4 months
2	1	1.5	6	0	Stable (neuro): 12.0 months
3	2	2.25	9	0	Stable (breast): 1 month
4	1	3.5	14	0	None
5	6	5.25	21	1: gr. 3 diarrhea	Stable (sarcoma): 6.5 months
6	3	6.56	26	0	Stable (renal): 8.0 months
7	6	8.25	33	1: gr 3 diarrhea	Stable (sarcoma): 5.5 months

iii) Development of 500 mg huanglian capsules and producing sufficient quantity to complete the phase I clinical trial and to conduct the phase II clinical trial in advanced breast cancer:

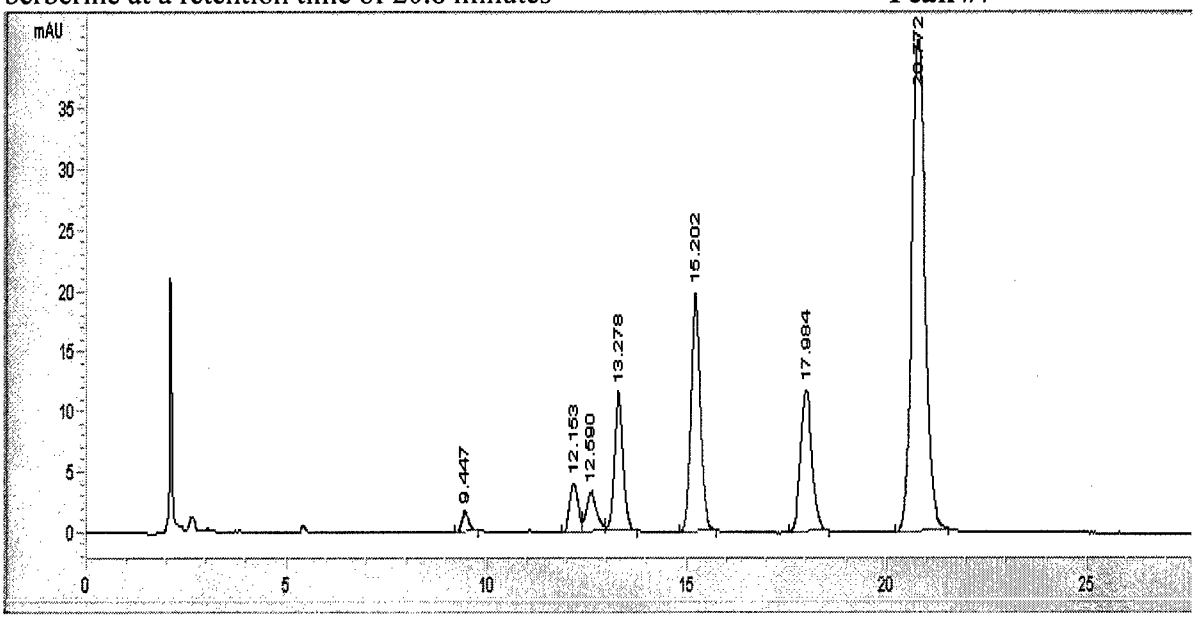
The immediate plan is to complete this phase I study of huanglian, so as to define the MTD and then test this in phase II clinical trials. We are especially encouraged by stable disease in patients with advanced cancers (breast, renal, sarcoma, and neuroendocrine tumors) who were progressing either under observation or on chemotherapy. These patients had no other treatment options at the point of study entry.

It is conceivable that for huanglian we may not be able to define an MTD with standard grade 3 and 4 dose-limiting toxicity. Instead the highest non toxic dose may be determined by the number of capsules consumed at any one time. In fact, if we did escalate to our next cohort in the current formulation of 250 mg capsules, patients would be taking 41capsules/day, divided in 4 doses. This would represent a pill count which will exceed that which a patient can take at any given time.

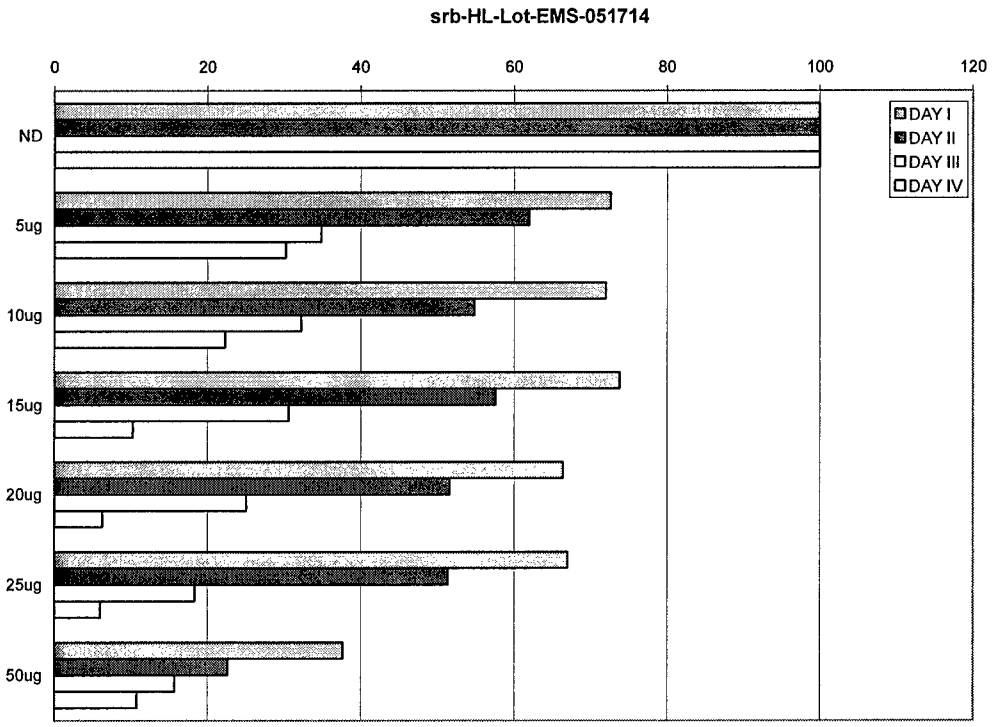
In order to address this, we are in the process of making 500 mg (rather than 250 mg) huanglian capsules for future clinical trials. Phoenix Laboratories, our outside contractor, has agreed to make these additional capsules at no cost to our institution. Without even a change in capsule size or formulation, we can package an additional 250 mg of huanglian into each capsule. We hope to obtain one lot of huanglian that should be sufficient to complete the phase I clinical trial and to conduct the phase II study in patients with metastatic breast cancer. Towards this end, we have tested several new lots of huanglian root for biological activity and chemical composition by HPLC. According to our protocol specifications the huanglian extract must have 7 peaks, which are within 5 to 10% of the original composition, and the predominant berberine peak (peak #7) must constitute 25%±5% of the whole extract. In addition, the huanglian extract must suppress the growth of the gastric cancer cell line, MKN-74, by at least 50% at huanglian extract concentrations of 5 to 10 μg/ml.

In the last 4 to 6 months we have tested a series of such extracts. The initial extracts were obtained from "huanglian" root obtained from Chinese food stores in the New York metropolitan area. All carried the label as being huanglian root. However, none of these had biologic activity comparable to the extract used in the clinical trial and were not therefore were not tested for biochemical composition. We next turned to suppliers in China who could send us huanglian root directly to our center. Three huanglian samples were sent to us. Though all three had comparable biologic activity, only one of these (labeled EMS) had the HPLC profile that exactly fit our definition for huanglian, had the necessary 50% biological activity against the MKN-74 cells, and contained sufficient berberine (20.5% of total weight) that was within the 25%±5% allowable for clinical use. These results are summarized below:

HPLC: Seven major components are identified by HPLC and the major component is berberine at a retention time of 20.8 minutes **Peak #7**

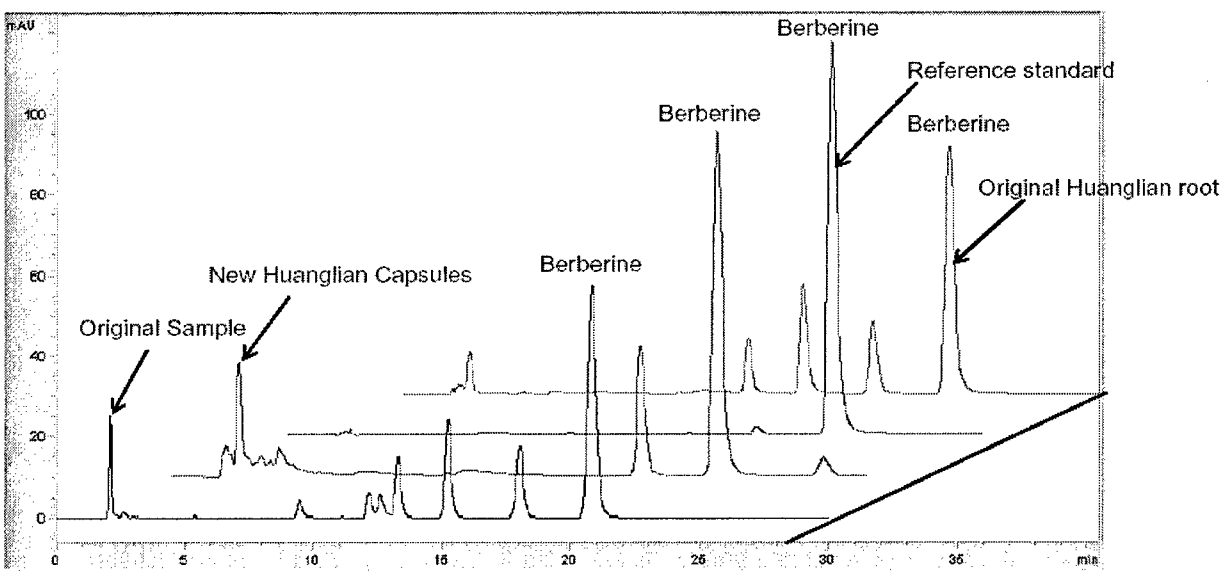


SRB Cell Proliferation Assays with increasing concentrations of huanglian extract: confirming inhibition of cell growth by at least 50% with continuous exposure of the herb to the MKN-74 cells for 3 (III) to 4 (IV) days. Results are shown for 5 to 50 μg/ml concentrations of huanglian extract and exposure times of 1, 2, 3, and 4 days.



The FDA has reviewed this data and has approved this lot of huanglian for clinical use. This information has been previously forwarded to the DOD for review. An amended protocol to allow us to use the 500 mg capsules has been approved by the MSKCC IRB (9/29/04, protocol version 7) and copy of the approved protocol with appropriate documentation has been forwarded to the DOD for review. The plan is to first retest the 8.25 gram dose (cohort 7) with the 500 mg capsule (16 capsules/day in 4 divided doses) to confirm safety and then begin dose escalation.

Based on this data we purchased 300 kg of huanglian from China. This material was forwarded to Phoenix Laboratories in the United States for the huanglian extraction process to produce approximately 100,000 capsules suitable for clinical use. We had used this laboratory group previously for this procedure and it had produced high quality huanglian, which met all of our criteria for clinical grade material. We have now completing retesting of the huanglian extract packaged as 500 mg capsules. Unfortunately, the new material does not meet our specifications and in fact contains 2 (not 7) peaks with only 11% (not 20 to 25%) berberine:



As shown above, the "new huanglian capsules", obtained after extraction, shows only 2 HPLC peaks with one of the two being berberine. This contrasts with our original huanglian obtained at the start of the clinical trial and the original huanglian root obtained before the initiation of the current extraction process (each with 7 peaks by HPLC with the dominant peak being berberine). We are now attempting to confirm these results. But, if confirmed, these results would indicate that the extraction process, which is necessary to prepare the herb for packaging in the capsules, severely affected the huanglian composition. This would suggest that we now need to go back to our supplier, identify and obtain another lot of huanglian from China, and again extract it for clinical use. We are working with Phoenix Laboratories to identify the failure in the production process. If we are not satisfied in identifying the problem, we are currently in the process

of identifying another laboratory which can do the extraction and packaging of the capsules.

Conclusions

Even with these set backs in obtaining suitable material for clinical use, we do believe that we can compete the phase I clinical trial in a timely fashion and still conduct the phase II clinical trial in patients with metastatic breast cancer. However, this process of drug development does illustrate the complexities of bringing botanical medicine into the area of clinical cancer therapy. Ensuring a reliable source of material that can be reproducibly called huanglian is critical for the success of this program. Our efforts clearly have implications for other investigators attempting to develop botanical medicines for breast cancer therapy. In order to do this in a meaningful and reproducible manner, it will be necessary to establish and maintain strict criteria so as to produce an "acceptable" drug product that is suitable for clinical cancer use. However, as we have learned, this is not easy, and even small deviations can result in the production of material that fails to meet these standards. Nevertheless, despite this apparent set back in huanglian production, we still believe we can identify a new lot of herb, such that we complete the two proposed projects, the phase I clinical trial of single agent huanglian (early 2005) and then a phase II clinical trial of huanglian in the treatment of metastatic breast cancer. Our initial plan was to conduct a combination study with paclitaxel in this patient population. However, in view of the interest and use of botanical medicines by patients with breast cancer, we believe a single agent trial in chemotherapy refractory breast cancer would be reasonable at this time. This study will be initiated as soon as we complete the phase I clinical trial with the 500 mg capsules of huanglian.